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ETIO PATHOGENESIS OF AUTOIMMUNITY

Adverse events following immunization with vaccines containing adjuvants

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Abstract A traditional infectious disease vaccine is a preparation of live attenuated, inactivated or killed pathogen that stimulates immunity. Vaccine immunologic adjuvants are compounds incorporated into vaccines to enhance immunogenicity. Adjuvants have recently been implicated in the new syndrome named ASIA autoimmune/inflammatory syndrome induced by adjuvants. The objective describes the frequencies of post-vaccination clinical syndrome induced by adjuvants. We performed a cross-sectional study; adverse event following immunization was defined as any untoward medical occurrence that follows immunization 54 days prior to the event. Data on vaccinations and other risk factors were obtained from daily epidemiologic surveillance. Descriptive statistics were done using means and standard deviation, and odds ratio adjusted for potential confounding variables was calculated with SPSS 17 software. Forty-three out of 120 patients with moderate or severe manifestations following immunization were hospitalized from 2008 to 2011. All patients fulfilled at least 2 major and 1 minor criteria suggested by Shoenfeld and Agmon–Levin for ASIA diagnosis. The most frequent clinical findings were pyrexia 68 %, arthralgias 47 %, cutaneous disorders 33 %, muscle weakness 16 % and myalgias 14 %. Three patients had diagnosis of Guillain–Barre syndrome, one patient had Adult-Still's disease 3 days after vaccination. A total of 76 % of the events occurred in the first 3 days post-vaccination. Two patients with previous autoimmune disease showed severe adverse reactions with the reactivation of their illness. Minor local reactions were present in 49 % of patients. Vaccines containing adjuvants may be associated with an increased risk of autoimmune/inflammatory adverse events following immunization.

Keywords Adjuvant · Vaccines · Autoimmunity · Aluminum · Thiomersal · Syndrome

Introduction

Adjuvants have been used for decades to improve the immune response to vaccine antigens. Adjuvant is originated from the Latin word "adjuvare" which means "help" in English to enhance the immunologic responses when given together with antigens. The beginning of adjuvant was mineral oil which enhanced the immune response when it was given with inactivated *Salmonella typhimurium* [1]. Aluminum salt was used to precipitate diphtheria toxoid and increased level of antibody response was demonstrated when administered with alum-precipitated antigens. Since 1930, aluminum salt has been used as diphtheria-tetanus-acellular pertussis (DTaP) vaccine adjuvant. Many candidates were tested for adjuvant activity but only aluminum salt is allowed to use for human vaccines [2]. New adjuvant MF59, oil-in-water emulsion type, was developed for influenza vaccine for elderly (Fluad), and series of AS adjuvant are used for hepatitis B, pandemic flu and human papilloma virus vaccines. Oil-

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adjuvanted influenza pandemic vaccines induced higher antibody response than alum-adjuvanted vaccine with higher incidence of adverse events, especially for local reactions [3, 4]. Alum-adjuvanted whole virion inactivated H5N1 vaccine was developed in Japan, and it induced relatively well immune responses in adults. When it applied for children, febrile reaction was noted in approximately 60 % of the subjects, with higher antibodies. Recent investigation on innate immunity demonstrates that adjuvant activity is initiated from the stimulation on innate immunity and/or inflammasome, resulting in cytokine induction and antigen uptake by monocytes and macrophages [5, 6].

A new syndrome was recently introduced and termed ASIA "Autoimmune (Auto-inflammatory) Syndrome Induced by Adjuvants." This syndrome includes a spectrum of immune-mediated diseases triggered by an adjuvant stimulus such as chronic exposure to silicone, tetramethylpentadecane, pristane, aluminum and other adjuvants [7].

Thus, in the current study, we analyzed the clinical characteristics of patients who developed post-immunization disease, aiming to find common denominators and the relevancy of the ASIA preliminary criteria of this cohort.

Patients and methods

The medical records of 120 patients who experienced an adverse event following immunization (AEFI) were evaluated. We defined an AEFI as any untoward medical complication that follows immunization 54 days prior to the adverse event, according to the official Mexican standard: NOM-017-SSA2-1994. The application of vaccines took place in several health centers in Guadalajara, Mexico between 2008 and 2012, and was performed according to Mexican General Health Law NOM-036-SSA2-2002 [8, 9]. The patients who we included in the present cross-sectional study were only who experienced the emergence of a new non-specific inflammatory response (fever, myalgia, arthralgia), immune-mediated sign or symptom following vaccination and who were diagnosed by an immune disease specialist. Data about vaccines and their adjuvants used in our population and other risk factors were obtained from daily epidemiologic surveillance. We considered immunization error if inappropriate transportation or storage, failure to adhere to recommended schedule, use of expired product or wrong diluents, incorrect dosage, injecting equipment, sterile technique, route or site of injection. This study was approved by local ethics committee and according to the ethical guidelines of the declaration of Helsinki.

The medical records of the patients were evaluated for demographics, past medical history, dates and number of inoculations. We collected the information of local and immediate adverse events, as well as clinical manifestations and their temporal relation to the vaccines doses. We also recorded all available data of blood tests, imaging modalities, tissue biopsy and treatments. The preliminary ASIA criteria were applied to each patient. Statistical analysis with application of descriptive statistics was done using means and standard deviation, and odds ratio adjusted for potential confounding variables was calculated with SPSS 17 software.

Results

Forty-three patients, out of 120, fulfilled with the criteria of moderate or severe AEFI patients were included, 17 (40 %) patients were adults and 26 (60 %) were in childhood age, 25 (58 %) were females. Their mean age was 14 ± 19.8 years (range from 1 to 82 years old). Familial history of allergic and neurological diseases was documented in 28 % of our patients, and personal history of allergic and neurological diseases was documented in 30 % of our cohort. The total numbers of inoculations were 121 with a mean of 3.6 for each patient (range 1–9). The average period from immunizations to onset of symptoms was 6 days (ranging from <12 h to 39 days) and ratio female–male was 1:1.2. The vaccination error was made in 6 (14 %) of the patients. Moderate and severe AEFI were diagnosed in 27 (63 %) and 16 (37 %), respectively. There was more risk in child population for AEFI with an OR 6.4.

The most frequent vaccines applied, according to immunization schedule recommended for the Mexican Secretariat of health, and their adjuvants are grouped in Table 1.

Minor local reactions were documented in 21 (49 %) of the patients, including redness 60 % and pain at site of inoculation 85 %. Neurological manifestations were found

Table 1 Vaccines and adjuvants

Vaccine	Adjuvant
MR booster	Neomycin + sorbitol + albumin
HepB booster	Aluminum salt + thiomersal
Flu H1N1 + H3N2	Thiomersal + NaCl + KCl + MgCl
DTaP/HepB/Hib	Aluminum salt + phenoxyethanol
Flu H1N1	Squalene
IPV	MgCl
Flu vaccine	Thiomersal + NaCl + KCl + MgCl
DTaP booster	Aluminum salt + thiomersal + formol
PCV S23 vaccine	Thiomersal + phenol
MMR	Neomycin + sorbitol + albumin
DTaP	Aluminum salts + thiomersal

in 21 (49 %) of the group, somnolence 7 (16 %), seizures 6 (14 %), headache 4 (9.3 %), peripheral neuropathy 4 (9.3 %), hypotonia 4 (9.3 %), paralysis 4 (9.3 %). Psychiatric disturbances were mainly irritability 10 (24 %). Mucocutaneous manifestations were reported in 14 (33 %) of the patients, rash 8 (19 %), sicca syndrome 4 (9.3 %), petechiae 5 (12 %), skin pigmentation 1 (2.3 %), purpura 1 (2.3 %). Musculoskeletal complaints were documented in 26 (60 %) of cases, arthralgia 20 (47 %), myalgia 6 (14 %). Gastrointestinal complaints were found in 35 % of patients, abdominal pain 7 %, diarrhea 9 %, nausea 9 %, vomiting 7 % and non-immune pancreatitis 2.3 %. General symptoms were reported in 70 % of cases, fever 68 %, lymph node enlargement 23 %, muscle weakness 16 % and angioedema 20 %.

We found anti-nuclear antibodies in 1 patient (2.3 %). No other autoantibodies were detected. Decreased in nerve conduction velocity in four patients demonstrated demyelinating neuropathy. Cerebral MRI in one patient was the evidence for diffuse demyelinating lesions.

ASIA preliminary criteria were applied to all 43 patients, of whom 60 % were children and 40 % were adults. All patients fulfilled at least 2 major and 1 minor criteria suggested by Shoenfeld and Agmon–Levin for ASIA diagnosis.

The numbers of AEFI and seriousness for each vaccine applied are depicted in Table 2.

In this study, various post-vaccination diseases were diagnosed as specified in Table 3.

All patients fulfilled at least 2 major and 1 minor criteria suggested by Shoenfeld and Agmon–Levin for ASIA diagnosis. Typical clinical findings of ASIA in this study included myalgia, myositis or muscle weakness 30 %, arthralgia and/or arthritis 47 %, neurological manifestations 48 %, fever 68 %, dry mouth 9.3 %.

Table 2 Vaccines and severity of AEFI

Vaccine	AEFI	Moderate	Severe	*P*
Flu H1N1 + H3N2	12	7	5	0.485
Flu H1N1	7	4	3	1
IPV vaccine	6	3	3	0.655
PCV S23 vaccine	3	1	2	0.545
MMR	2	0	2	0.133
Flu vaccine	5	4	1	0.635
BCG	6	5	1	0.386
DTaP + IPV + HepB	8	7	1	0.113
DTaP	1	1	0	1
DTaP booster	4	1	3	0.137
HepB booster	23	14	9	1
MR booster	30	19	11	0.587

Table 3 Specific post-vaccination diseases

Diagnosed diseases	Number of patients (%)
Neurological diseases[a]	6 (14)
Auto immune/inflammatory diseases[b]	4 (10)
Infections[c]	2 (5)
Systemic vasculitis[d]	2 (5)

[a] Neurological diseases include: Guillain–Barre syndrome, encephalomyelitis and vascular cerebral disease

[b] Autoimmune diseases include: Diabetes mellitus, rheumatoid arthritis, inflammatory bowel disease and Adult-onset Still's disease

[c] Infections: Gastroenteritis, local abscess

[d] Vasculitis: Kawasaki disease, severe cutaneous small-vessel vasculitis

Discussion

Most successful vaccines have been derived empirically and are capable of inducing robust T- and B-cell immunity without any adjuvant additives. Emerging evidence suggests that such live vaccines induce innate immune activation via a range of stimuli, including ligands specific for Toll-like receptors, which in effect, serve as their own adjuvants. In contrast to these live vaccines, subunit vaccines need to be supplemented with adjuvants to boost their immunogenicity. Interest in vaccine adjuvants has been growing rapidly for several reasons. Vaccine manufactures and public health authorities have established ambitious goals for enhancing present vaccines and for developing new ones, and new vaccine candidates have emerged over the past years against infectious, allergic and autoimmune diseases and also for cancer and fertility treatment [10].

Adjuvants include environmental compounds that have been recognized for decades as autoimmunity inducers in different animal models and that are used in the pharmaceutical industry to develop antigenicity and to decrease the cost of vaccine production. Adjuvants, as it is already known, can trigger the development of inflammatory or autoimmune illnesses in genetically susceptible humans [10]. Among this large group, which includes infectious fragments, hormones, aluminum, silicone, mercury, squalene has recently been highlighted, it is a natural oil obtained from shark tissue and constitutes one of the principal adjuvants used in the anti-influenza vaccine [11]. In the well-designed study published by Katzav A et al., they demonstrated the ability of adjuvant to induce autoimmunity in prone mice. When adjuvants were injected into transgenic Factor V Leiden mice, pathogenic anti-phospholipid antibody production was induced [12].

Our results suggest that vaccines containing adjuvants may be associated with an increased risk of autoimmune/inflammatory adverse events following immunization. Adjuvants that were used in the vaccines applied to our

cohort are mainly aluminum salts, thiomersal and squalene, other were sorbitol, albumin and neomycin. Aluminum salts-containing vaccines were the most associated with AEFI, some others had similar data [12, 13].

We analyzed the data of 43 patients most of them were female. The latency period of onset of autoimmune symptoms in our group was shorter than the traditionally 3–6 weeks, with the most frequency in the first week post-inoculation. Current medical literature documents variable latency periods, ranging from 3 weeks to 12 months or even several years for the beginning of the immune-mediated damage [14, 15]. The most of our patients received more than one vaccine and vaccines containing more than one adjuvant. We were especially interested in patients who received vaccine boosters because they had more AEFI, mainly with HepB and MMR boosters. The childhood population was more susceptible to develop adverse immune response OR 6.4, the children are more exposed to vaccines because the immunization schedule, and that is why we speculate that children are more exposed to adjuvants. We did not consider that our cohort had more genetic susceptibility, because personal and familial background was irrelevant. Although by the design of the study, we possibly have an underestimation of the true prevalence of autoimmunity among family individuals.

Aluminum-associated neuronal toxicity has been well documented in humans [16]. Aluminum salts (alum) have been used as adjuvants with great success for almost a century and have been particularly effective at promoting protective humoral immunity. However, alum is not optimally effective for diseases, where cell-mediated immunity is required for protection [17, 18]. While the total concentration of aluminum at the injection site will be high, the availability of cytotoxic Al (aluminum) is unlikely to be high enough to induce necrotic cell death, and it is important to consider that infiltrating phagocytes will find an unlimited diet of particulate Al adjuvant and will "eat" until they die, thereby releasing various damage-associated molecular patterns (DAMPs). The environment rich in Al adjuvant and DAMPs would increase the possibility of activation of the Nalp3 inflammasome, and the production of IL-1β, and thus, induction of inflammation and increased recruitment, activation and maturation of immune cells [18–23]. Thiomersal, also called thimerosal, is an ethyl mercury derivative used as a preservative to prevent bacterial contamination of multi-dose vaccine vials after they have been opened. Thimerosal is one of the most important organic mercury compounds human populations are exposed to. It has toxic effect on several cell lines, and it also induces programmed cell death in in vitro experiments. Association is suggested between application of thimerosal-containing vaccines and the occurrence of neurodevelopmental disorders, like autism and tics, also with systemic vasculitis [24–27]. While specific recommendations were made to eliminate thimerosal from vaccines, consistent evidence is still lacking for an association of exposure and disease [25, 26]. No cases of autism were detected in our cohort. Currently in our country at least 6 vaccines included in the immunization schedule contains thiomersal. The clinical manifestations reported in this 43 patients involved different body systems such as mucocutaneous, neurological, musculoskeletal and constitutional symptoms are comparable with the data in medical literature [12–14, 28], our cohort had lower incidence of diseases diagnosed such as neurological illness or autoimmune disorders. We believe that the under-registration of clinical data on medical records is because most of post-immunization complaints are considered not relevant by patient and physician moreover some signs and symptoms could be confounded with adverse events related to the vaccine itself.

All patients fulfilled at least 2 major and 1 minor criteria suggested by Shoenfeld and Agmon–Levin for ASIA diagnosis. The ASIA syndrome includes four major and four minor criteria and we can classify as having the syndrome if the patient has two major criteria or one major and two minor criteria.

Some limitations to our study are the cross-sectional design (retrospective) and the lack of control group in order to prove causal correlation between vaccine and clinical manifestation. Our sample of patients was small and selection bias could have occurred due to our state referral center and by the pre-selection by immune specialist. Also, not all the subjects underwent biopsies or MRI. This is to our knowledge the first case series in Mexico that suggests a probable association between vaccine adjuvants and immune-mediated diseases. Several aspects have been pointed out about our local health regulations respect to immunizations, the use of multiple vaccines and their adjuvants. We recommend the systematic clinical search of the "typical" clinical findings for ASIA in patients, children or adults, with recent immunizations. Further prospective studies are needed for clarification of risk factors and causality of the additive compounds termed adjuvants.

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